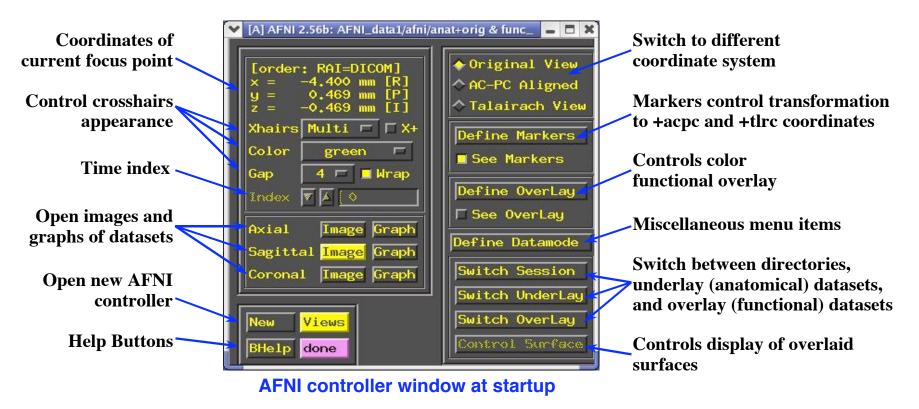
Using **AFNI** Interactively

- Start AFNI from the command line
 - * afni reads datasets from the current directory
 - * afni dir1 dir2 ... reads datasets from directories listed
 - * afni -R reads datasets from the current directory and from all directories below it
- AFNI reads the file named .afnirc from you home directory, if it is present
 - ★ This file is used to change many of the defaults (cf. File README.environment)



- Miscellaneous features of the AFNI controller window:
 - ★ xyz-coordinate display in upper left corner shows current focus location
 - ♦ By default, the coordinates are in <u>RAI</u> order (from the <u>DICOM</u> standard):
 - $\rightarrow x = \underline{R}ight$ (negative) to Left (positive)
 - $\rightarrow y = A$ nterior (negative) to Posterior (positive)
 - $\rightarrow z = Inferior$ (negative) to Superior (positive)
 - ♦ This display order can be changed to the neuroscience imaging order <u>LPI</u>:
 - $\rightarrow x = \underline{\mathsf{L}}$ eft (negative) to Right (positive)
 - $\rightarrow y = Posterior$ (negative) to Anterior (positive)
 - \Rightarrow z = Inferior (negative) to Superior (positive)
 - ★ The [Bhelp] button: when pressed, the cursor changes to a hand shape; use it to click on any AFNI button and you will get a small help popup
 - → AFNI also has 'hints' (AKA 'tooltips')
 - ★ Press the [New] button to open a new AFNI controller
 - * Used to look at more than one dataset at a time
 - ↑ [Define Datamode] ⇒ [Lock] can be used to lock controllers together by coordinates
 - → All viewing windows within a controller are always locked together
 - ♦ Press the [Views] button to close/open the control panel at right

- ★ Press the [done] button twice within 5 seconds to exit AFNI
 - ♦ The first button press changes 'done' to 'DONE'
 - → Fail to press second time in 5 seconds and it changes back to 'done'
 - Whatever you do, don't press a mouse button in the blank area to the right of [done]
 - → I won't be responsible for the consequences
- ★ The [Switch] buttons let you control which datasets are being viewed
 - [Switch Session] controls which directory datasets are drawn
 from
 - [Switch Underlay] control the background (grayscale) dataset --anat dataset usually goes here
 - → Current anat dataset determines the resolution of and 3D region covered by image viewers
 - [Switch Overlay] controls the overlay (color) dataset --- func dataset usually goes here
 - → Func datasets will be interpolated -- if needed -- to anat resolution, and flipped -- if needed -- to anat orientation
 - Current datasets are named in AFNI controller titlebar

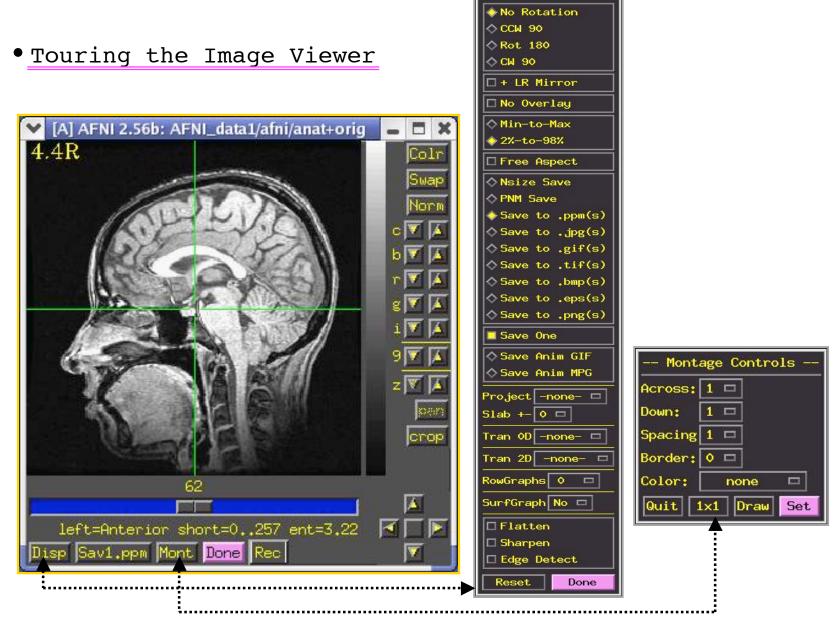


Image viewer; **Disp** and **Mont** control panels

- * Crosshairs show the current focus location
 - Also show the cut planes for the other image viewers
 - When using image montage, other viewers show multiple crosshairs
 - Can control crosshair color and gap size from main AFNI controller
- ★ Slider below image lets you move between slices
 - Left-click and drag 'thumb' to move past many slices
 - ♦ Left-click ahead or behind thumb to move 1 image at a time
 - → Hold click down to scroll continuously through slices
 - Middle-click in 'trough' to jump quickly to a given location
- ★ Vertical intensity bar to right of image shows mapping from numbers stored in image to colors shown on screen
 - Bottom of intensity bar corresponds to smallest numbers displayed
 - Top corresponds to largest numbers displayed
 - ♦ Smallest-to-largest display range is selected from [Disp] control panel
 - → or from hidden popup menu on intensity bar
 - All image viewers from all AFNI controllers use the same intensity bar
 - ⇒ unless AFNI is started with the <u>uniq</u> command line option, in which case each AFNI controller's viewers have independent intensity bars
 - → but all image viewers from the same controller always share the same intensity bar

★ Buttons at right

- ♦ [Colr] changes grayscale to color spectrum, and back
- ♦ [Swap] swaps top of intensity bar with bottom
- ♦ [Norm] returns the intensity bar to normal (after you mess it up)
- ♦ [c] controls contrast
- ♦ [b] controls brightness
 - Useful combination [c] ▲ 2-3 times, [b] ▼ 2-3 times
- ♦ [R] rotates the intensity bar (useless, but very fun)
- [g] changes the gamma factor (nonlinearity) for the intensity bar
- ♦ [I] changes the size of the image in the window
- ♦ [9] changes the opacity of the color overlay
 - → This control only present for X11 TrueColor displays
- ★ At bottom right, the arrowpad controls the crosshairs
 - Arrows move 1 pixel in that direction for that window
 - Sagittal is same as Axial
 - Central button closes and opens crosshair gap
 - ♦ Items on AFNI controller (below xzy display) also alter crosshairs
 - → Can change color, gap size, ...

- ★ Buttons along bottom provide various services
 - ♦ [Disp] controls the way images are displayed and saved
 - → Pops up its own control window: most controls change image immediately
 - → Orientation controls at top allow you to flip image around
 - → [No Overlay] lets you turn color overlays off (crosshairs; function)
 - \rightarrow [Min-to-Max] \Rightarrow intensity bar is data min-to-max
 - ⇒ [2%-to-98%] ⇒ intensity bar is smallest 2% of data to largest 98%
 - **►** [Free Aspect] lets you distort image shape freely
 - **Save panel**] controls how images are saved to disk:
 - All buttons off ⇒ saved image file contains slice raw data
 - [Nsize Save] \Rightarrow same, but images are 2^N in size
 - [PNM Save] ⇒ images are saved in PPM/PGM format (color/gray)
 - [Save to .xxx(s)] \Rightarrow saves image(s) to specified format
 - [Save One] ⇒ for saving montage
 - → [Tran OD] lets you transform voxel values before display
 - [Log10] and [SSqrt] useful for images with extreme values
 - → [<u>Tran 2D</u>] provides some 2D image filters (underlay only)
 - [Median 9] smoothing can be useful for printing images
 - → [Rowgraphs] lets you graph the voxel values from image rows
 - If you want columns, flip the image with [<u>CCW 90</u>]
 - → [Surfgraph] lets you graph the voxel values in a surface graph

- → Three extra imaging processing filters are provided at the bottom
 - [Sharpen] is sometimes useful for deblurring images
- → [Done] closes this control window
- ★ [Save] lets you save images from viewer to disk files
 - ♦ Warning: Images are saved as sent to the viewer, not as displayed
 - → Means that aspect ratio of saved image may be wrong (non-square pixels)
 - → Can fix this with [Define Datamode] ⇒ [Warp Anat on Demand]
 - [Save:bkg] means it will save the background image data itself, whatever the format it may be in (bytes, shorts, floats, complex numbers, RGB byte triples)
 - ♦ [Save:pnm] means it will save the displayed image in PNM format
 - → PPM for color, PGM for gray-only images
 - → You might have to convert this to some other format
 - → See AFNI FAQ #57 for instructions on image format conversion
 - ♦ [Sav1:xxx] means it will save the entire Montage in format "xxx"
 - → This is the <u>only</u> way to save a Montage layout (within AFNI)
 - [Save] options will only save single slice images (one or more)
 - ♦ [Save.xxx] means it will save the image in the "xxx" format
 - You can also set this using the hidden right-click popup on the [Save] Button
 - → Formats depend on presence of image conversion programs on your system

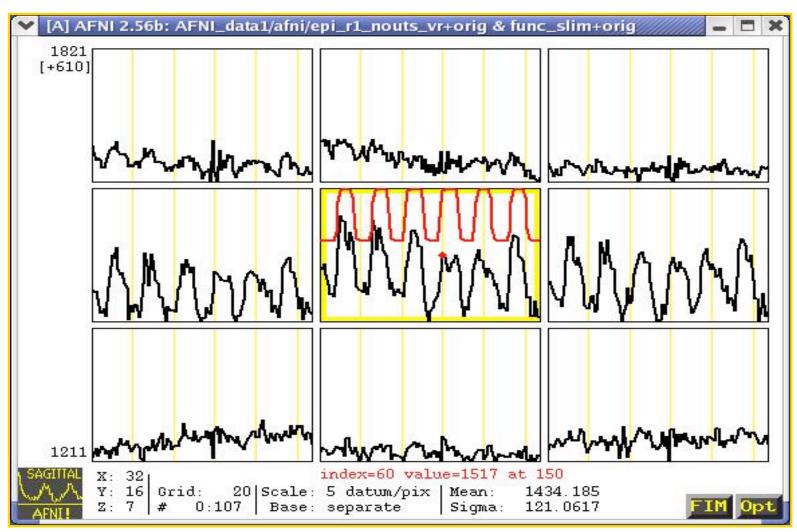
- ♦ After you press [Save], then it asks for a filename prefix
- ♦ Except for [Sav1.xxx], it then asks for 'from' and 'to' slice indexes
 - You can save many images this way
 - → Filenames are like are like ppm, for slice #37, ppm format
 - ⇒ [Sav1.xxx] immediately saves its one file after prefix is entered
- ★ [Mont] lets you display a rectangular layout of images (i.e., montage)
 - ♦ Pops up its own little control window
 - → Controls at top do nothing until an action is selected at bottom
 - ♦ [Across] and [Down] determine number of sub-images shown
 - ♦ [Spacing] determines how far apart the selected slices are
 - ightharpoonup Every n^{th} slice, for n = 1, 2, ...
 - → Multiple crosshairs in other image viewers will show montage slices
 - ♦ [Border] lets you put some blank pixels between sub-images
 - → [Color] lets you choose the color of the border pixels
 - ♦ At the bottom, the action controls cause something to happen:
 - [Quit] closes the Montage control window
 - → [1x1] changes Across and Down back to 1
 - [Draw] actually causes the montage to be drawn
 - → [Set] ⇔ [Draw] then [Quit]

- ★ [Rec] lets you record images for later Save-ing
 - ♦ So you can build a sequence of images from any set of AFNI controls
 - → Change color maps, functional thresholds, datasets, ...
 - ♦ Then save them to disk for animation, etc.
 - → If Unix programs whirlgif and/or gifsicle are installed on your system, AFNI can write GIF animations directly (e.g., for fun Web pages)
 - → If program mpeg_encode is installed, AFNI can write MPEG-1 animations
 - → Source code for these free programs is included with AFNI source code
 - ♦ [Rec] button pops down a menu that sets the record mode
 - → [Off] ⇒ recording is off
 - ► [Next One]
 ⇒ next image displayed is recorded, then goes back to [Off]
 - □ [Stay On] ⇒ record each image when displayed
 - → Controls below the line determine where in the recording sequence the saved images will be stored
 - Recorded images go into a new image viewer, with its own controls
 - → Its slider moves between recorded images
 - → [Kill] will delete an image from the recorded sequence
 - [Save] will save record images
 - Right-click on [Save] to bring up menu of format options
 - → [Done] to close the recorded image viewer

- ★ Hidden image popup menu (using Button 3 or right-click)
 - ♦ [Jumpback] lets you jump the focus position back to its last place
 - → For when you click in the wrong place and get lost
 - ♦ [Jump to (xyz)] lets you enter xyz-coordinates (in mm), and then the focus position will jump there
 - \Rightarrow External program 3dclust can generate xyz coordinates of interest
 - → Once you have +tlrc dataset, can jump to regions from Talairach atlas
 - ♦ [Jump to (ijk)] lets you jump to a particular voxel index location
 - ♦ [Image display] lets you turn control widgets on and off
 - → Can unclutter screen a little
 - → Useful if you want to make a screenshot
- ★ Hidden intensity bar popup menu
 - [Choose Display Range] lets you pick the range of numbers that are mapped to intensity bar colors
 - → Normally, each image is mapped to colors separately when it is displayed
 - Using Min-to-Max or 2%-to-98% from [Disp]
 - → If you want each image to be mapped the same, then must give bottom-totop values via this menu item (separate them with spaces)
 - → If you set third (optional) input 'ztop' to 1, values above 'top' are set to 0
 - → To restore normal auto-mapping, set 'bot' and 'top' both to 0

- ♦ [Choose Zero Color] lets you choose the color that is displayed for voxel values that are exactly 0
 - Can be useful for filling in regions that were set to 0 by some program
 - → For example, values below 'bot' from Choose Display Range (and above 'top' if 'ztop' was set to 1)
 - → Choose the 'none' color to return to normal display
- ♦ [Choose Flatten Range] is used to control the Flatten filter from the [Disp] control window
 - → This is almost useless --- don't bother to try it
- [Choose Sharpen Factor] is used to control the Sharpen filter
 from the [Disp] control window
 - → Larger values mean more sharpening (and more image graininess)
- ♦ [Plot Overlay Plots] turns overlay graphs on and off
 - → In future, will control overlay of cortical surface geometry
 - → This feature is experimental now, and not documented
- ♦ [Label] and [Size] controls display of slice coordinate overlay

• Touring the Graph Viewer



Graph Viewer with data (black) and reference waveforms (red)

- ★ Graph viewer takes voxel values from same dataset as image viewer
 - ♦ If dataset has only 1 sub-brick, graph viewer only shows numbers
 - ◆ To look at images from one dataset locked to graphs from another dataset, must use 2 AFNI controllers and [Define Datamode] ⇒ [Lock] on AFNI control panel
- ★ If graph and image viewer in same slice orientation are both open, crosshairs in image window change to show a box containing dataset voxels being graphed
- ★ Central sub-graph (current focus location) is outlined in yellow
 - Current time index is marked with small red diamond
 - Left-clicking in a non-central sub-graph moves that location to focus
 - Left-clicking in central sub-graph moves time index to that point
 - → Can also use [Index] control in AFNI controller
 - ♦ Right-clicking in any sub-graph pops up some statistics of its data
 - Left-clicking in icon (lower left corner) causes icon and menu buttons to disappear

 - → Left-clicking in same place will bring icon and buttons back

- ★ [Opt] menu buttons let you control how graphs appear
 - Many items have <u>keyboard</u> shortcuts
 - → Make sure you are typing in the correct window!
 - ♦ [Scale] changes scale of graphs
 - → Mapping from voxel values to screen pixels
 - → **Down** [] shrinks graphs vertically; **Up** [+] expands them
 - → Auto [a] makes AFNI pick a nice scale factor
 - → [Choose] lets you pick exact scale factor
 - Can choose positive values = pix/datum or negative = datum/pix
 - pix/datum = number of screen pixels for each change of 1 in data
 - datum/pix = size of change in data for each screen pixel
 - → Current scale factor is shown below graphs
 - → Scale factor does *not* change when you resize graph, change matrix, etc.
 - You usually have to auto-scale [a] afterwards
 - ♦ [Matrix] changes number of sub-graphs
 - → **Down** [m] and **Up** [M] decrease and increase number
 - → [Choose] lets you pick number exactly
 - Alternative: keyboard [N], type number, then [Enter] key
 - Range of allowable matrix size is 1..21

- ♦ [Grid] lets you change spacing of vertical grid lines
 - Useful for showing regular timing interval (e.g., task timing)
 - ▶ Down [g] and Up [G] decrease and increase spacing
 - → [Choose] lets you pick number exactly
 - Current grid spacing is shown below graphs
 - → [Pin Num] lets you pick the horizontal length of the sub-graph
 - Default length is number of sub-bricks in dataset
 - Make it longer ⇒ graphs end before window
 - Make it shorter ⇒ graphs are truncated
 - Useful when switching between datasets of different lengths
 - Set this to 0 to get back to default operation
 - Current number of time points is shown below graphs
 - **→ Horz** [h] will put in a dashed line at the y = 0 level in sub-graphs
 - Only useful if data range spans negative and positive values!
- ♦ [Slice] lets you change slices
 - → Down [z] and Up [Z] move one slice
 - Can also choose slice directly from menu
 - → Current voxel indexes are shown below graphs
 - Corresponds to [Voxel Coords?] Display in AFNI controller

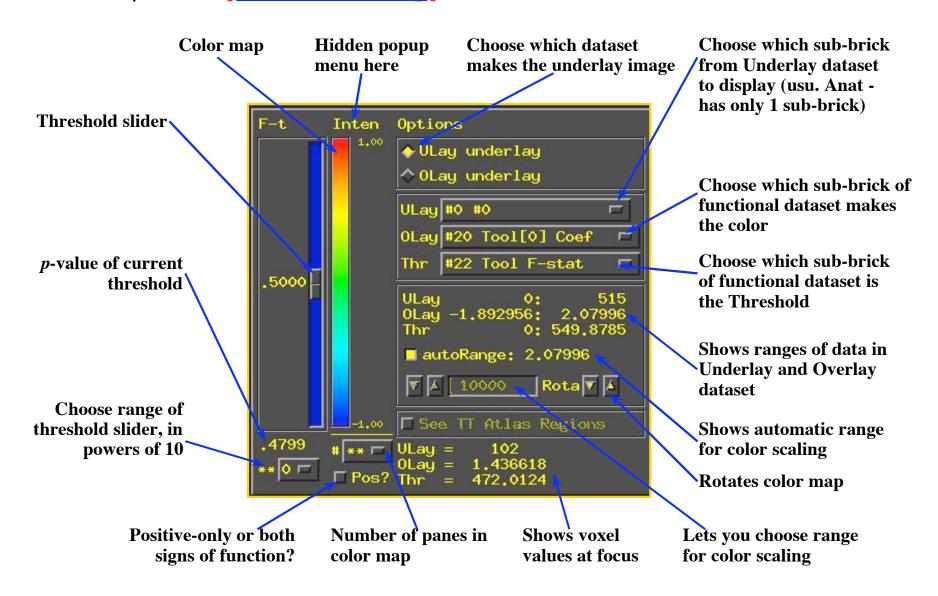
- ♦ [Colors, Etc.] lets you alter the colors/lines used for drawing
 - → Lines used for sub-graph frame boxes, grid lines, data graphs, FIM orts/ideals, and double plots can have color changes and be made thicker
 - Grid color is also used to highlight central sub-graph
 - → Can choose to graph curves as lines, points, or both together
 - → Can change color of background and text
 - → Can change gap between sub-graph boxes
- ♦ Baseline [b] changes how the sub-graphs are plotted
 - → All sub-graphs have same scale factor, to convert values into vertical pixels
 - ⇒ Baseline is value that gets plotted to bottom of sub-graph
 - → Individual: all sub-graphs have different baselines
 - Baseline = smallest value in each displayed time series
 - This can be confusing; same vertical location doesn't mean same value
 - Shown below graphs as Base: separate
 - → Common: all sub-graphs shown at any one time get same baseline
 - Baseline = smallest value in all displayed time series
 - Shown below graphs as Base: common
 - Usually need to rescale [a] after changing baseline
 - → <u>Global</u>: all sub-graphs get same baseline even when spatial position changes
 - Set from [Baseline] ⇒ [Set Global] menu item
 - Default global level is smallest value in entire dataset

- → Range of central sub-graph is shown at left of graph region
 - Central bottom (baseline) value is shown at lower left
 - Upper left shows value at top of central sub-graph box
 - Number in [brackets] shows data range of one sub-graph box's height
 - If baselines are separate, bot/top values only apply to central sub-graph
- ♦ Show text? [t] allows you to see text display of values instead of graphs
- ♦ Save PNM [S] lets you save a snapshot of window to a PNM image file
- ♦ Write Center [w] lets you write data from central sub-graph to a file
 - → File is in ASCII format ⇒ can be imported into other programs
 - ⇒ Filename is of form xxx yyy zzz.suffix.1D (using voxel indexes)
 - ⇒ Suffix is chosen using [Set 'w' suffix] button
- ♦ [Tran OD] and [Tran 1D] let you transform the data before graphing
 - → [Log10] and [SSqrt] useful for images with extreme values
 - → [Median3] and [OSfilt3] are for are for smoothing time series
 - → Other choices are functions controlled by/from plugins
 - [Double Plot] lets you plot output of [Tran 1D] and original data together
 - Color of transformed data from [Dplot] on the [Colors, Etc.]
 menu
 - [Dataset#2] transformation lets you plot two datasets together

- \diamond [X-axis] menu lets you choose how graph x-axis is chosen
 - → Default: *x* is linear in time
 - ⇒ Can instead choose x from a .1D format file from disk
 - → Useful only in very limited circumstances
- ♦ Done [q] closes the graph viewer window
- * **Keystrokes** in graphs that have no menu items are:
 - ♦ [<] moves time index down by 1</p>
 - ♦ [≥] moves time index up by 1
 - ♦ [1] moves time index to beginning (time index = 0)
 - ♦ [1] moves time index to end
 - ♦ [L] turns off/on the AFNI logo in the corner
- * [FIM] menu controls interactive functional image calculations
 - Not documented here; see 'Educational materials' pages at AFNI Web site

• Brief Tour of the Functional Color Overlay Controls

★ Open with [Define Overlay] button on AFNI controller



AFNI Plugins

- Plugins are modules that attach themselves to AFNI and add some interactive capabilities to the GUI program
- There is a (somewhat dated) manual for writing plugins
- Useful plugins:
 - * 3D Registration:

Provides a GUI control for time series registration (same as 3dvolreg)

* Dataset Copy:

Copy a dataset (useful as a start for ROI drawing)

* Dataset NOTES:

Add arbitrary text notes to a dataset header

* Draw Dataset, Gyrus Finder:

Draw regions-of-interest (ROIs) on 2D slices

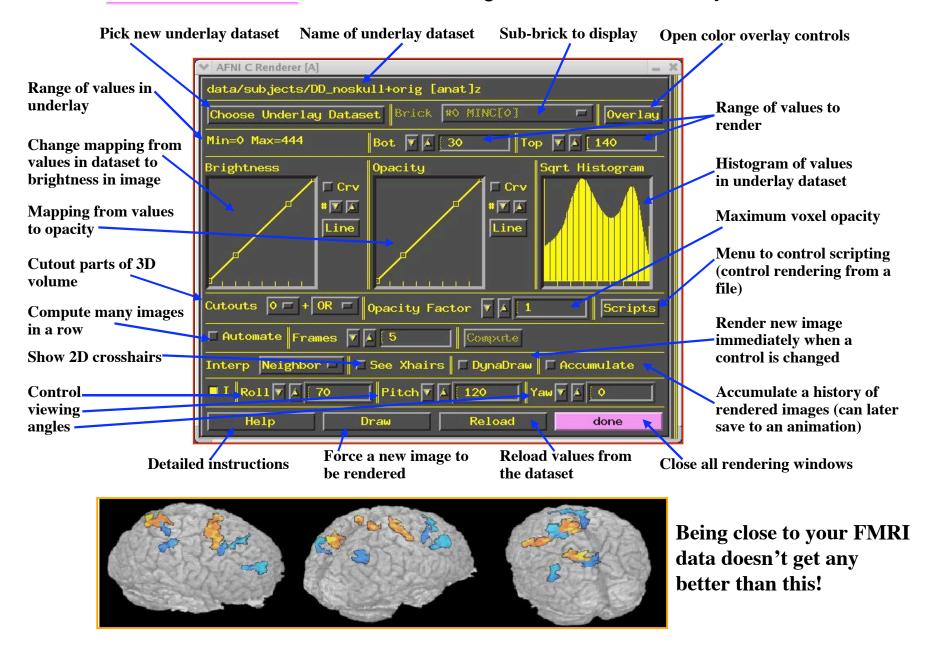
* Histogram:

Graph the histogram of a sub-brick, or some parts of it

* Deconvolution, Nlfit & Nlerr:

Do linear and nonlinear regression interactively on the dataset time series being displayed in a graph viewer

* Render Dataset: Volume rendering with functional overlays



Using **AFNI** in Batch Mode

- Batch mode programs are run by typing commands directly to the computer, or by putting these commands into text files (scripts) and later executing them
- Advantages of batch mode (over graphical user interface)
 - ★ Can process new datasets exactly the same way as previous ones
 - ★ Can link together a series of programs to produce custom results
 - ★ Programs that take a long time to operate are easier to 'fire and forget' from a script than if they had a GUI
 - ★ It's easier to write a batch mode program
- Disadvantages of batch mode
 - * Requires typing, rather than pointing-and-clicking
 - ★ Requires learning/remembering how a program works all at once, rather than (re)discovering it through a kinder gentler interface
 - ★ Many younger (born after 1970) researchers have virtually no experience with a command line interface, or anything like it
- Many significant AFNI capabilities are only available in batch mode programs
 - ★ This is especially true of functions that combine data from multiple datasets to produce new datasets

- The 3d* series of programs (generally) take as input one or more AFNI datasets, and produce as output one (or more) new AFNI datasets
- Time series activation analysis programs:

★ 3dfim, 3dfim+, 3ddelay

Variations on 'classical' correlation analysis of each voxel's time series with a single reference (ideal) waveform

* 3dDeconvolve:

Multiple linear regression and/or linear deconvolution to fit each voxel's time series to a mulit-dimensional signal model (similar models are found in SPM)

* 3dNLfim:

Nonlinear regression to fit each voxel's time series to an arbitrary functional model provided by the user

• <u>Time series utility programs</u>:

* 3dFourier:

Fourier domain filtering voxels time series

* 3dTcorrelate:

Compute correlation coefficient of 2 datasets, voxel-by-voxel

* 3dTsmooth:

Smooth voxel time series

★ 3dTqual, 3dToutcount:

Examine voxel time series for statistical 'outliers'

* 3dTcat:

Shift voxel time series to a common temporal region

★ 3dTstat:

Basic statistics on voxel time series

★ 3dvolreg:

Volume registration to suppress motion artifacts, and to align same-subject data from different scanning sessions

• Multi-dataset statistical operations:

* 3dttest:

Voxel-by-voxel t-tests

★ 3dANOVA, 3dANOVA2, 3dANOVA3:

1-, 2-, and 3-way voxel-by-voxel ANOVAs, including random effects and nested models

* 3dFriedman:

Voxel-by-voxel nonparametric statistical tests analogous to ANOVAs

★ 3dRegAna:

General linear regression models and tests derived therefrom

• Miscellaneous operations on datasets:

★ 3dAnatNudge:

Try to align high-resolution anatomical volume with low-resolution EPI volume

* 3dClipLevel:

Find the voxel value to threshold EPI volume so as to remove most of the non-brain tissue

* 3dIntracranial:

Strip the scalp and other non-brain tissue from a high-resolution T1weighted anatomical volume

* 3dMean:

Compute the mean of a collection of datasets, voxel-by-voxel

★ 3dmaskdump, 3dmaskave, 3dROIstats:

Extract values from datasets and write to ASCII files

* 3dUndump:

Take values from ASCII files and write into a dataset

★ 3dmerge:

Lots of options to edit datasets and combine them in multifarious and nefarious ways

★ 3dZeropad, 3dZcutup, 3dZcat, 3dZregrid:

Utilities to add/subtract/resample datasets in the slice (z) direction

*3daxialize:

Re-write a dataset in a new slice direction

* 3dcalc:

General purpose voxel-by-voxel dataset calculator

★ 3dresample, 3dfractionize:

Resample a binary mask dataset from one resolution to another

* 3drotate:

Rotate a dataset to a new orientation in space

★ 3dpc:

Extract principal components from a collection of datasets

* 3dWinsor:

Spatially filter a T1-weighted anatomical dataset to reduce noise and make the gray-white matter boundary a little more distinct

* 3dclust:

Find clusters of activated voxels and print out statistics about them

★ 3dExtrema:

Find local extrema (maxima or minima) in a dataset --- intended for functional activation maps